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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Bleaching of dairy products

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BLEACHING OF DAIRY PRODUCTS

Field of the invention

Milk and milk related products are part of human nutrition. Milk products contain several nutrients, which are consumed for the human health and growth. The whiteness of milk or milk related products affects the perception of its taste and mouthfeel. Whiteness plays an important role in for example cheese, butter oil, milk powder or whey products. For example for cheeses like Feta, Mozzarella, Ricotta and blue cheese, whiteness is considered desirable. In cheeses wherein milk from goat or sheep is at least partially replaced by cow's milk, the whiteness of the cheese might be a problem because of the β-caroteen that is present in cows milk.

For some cheeses natural colouring agents like annatto or β-caroteen are used as food colouring agents. However, this colouring agent will also be present in the whey. When this whey is further processed into for example baby formula, the colour of the whey product may be undesirable.

Several options are known to whiten milk or milk related products:

The use of TiO_2: titanium dioxide is an inorganic inert white pigment used in candies, chewing gum, tooth paste etc. and approved by FDA as food grade. It is added to cheese milk in concentrations of 0.02 – 0.05% depending on type of milk and season (S. Laakso & E-M Lilius., J. Agric. Food Chem., 1982, 30, 913-916). Buffalo milk is originally used in Mozzarella cheese making. Its quantity is by far not capable of covering today's Mozzarella demand and therefore Buffalo milk is at least partially replaced by cow's milk. However Buffalo milk does not contain β-carotene as cow's milk does. Addition of TiO_2 in prescribed amounts does not affect taste and texture on the cheese. However off-flavour development in Feta cheese by adding TiO_2 has been reported (F. Kosikowski & D.P. Brown. J. Dairy Sci. 52, No. 7 968-970).

Another option to whiten milk or milk related products is the use of Benzoyl peroxide. This agent is used in the manufacturing of Mozzarella, blue types and certain Italian cheeses (F. Statens & Hilleroed., Beret. - Statens Forsoegsmejiri., 1978, 232, 36) in concentrations of 0.001 – 0.002%. The application is time consuming as cream is usually separated from the cheese milk and treated with this agent at 63°C for 45– 120 min. The peroxide is highly inflammable and contact with the skin should be avoided. Benzoyl peroxide is also used in bleaching of waxes, soaps, fats (including butterfat) and

bread. Benzoyl peroxide destroys β-carotene to such an extent that cheese milk or cheese should be supplemented with vitamin A. Moreover it may produce off-flavours.

The use of chlorophyls has been described for cheese whitening (F. Statens & Hilleroed., Beret. - Statens Forsoegsmejiri., 1978, 232, 36). The chlorophyls absorb the yellow colour of the β-carotene resulting in a net decolouring of the cheese. The dosage of chlorophyl is very critical; a small excess of the chlorophyll results in an undesirable greenish colour of the cheese. In production, the dosage is the principle problem, which makes the use of chlorophyll un-attractive. In addition, questions have been raised about the long-term effects on humans.

Other methods to whiten cheese are process related. Microfluidization of the cheese milk or its separated cream resulted in produced Cheddar cheese with a whiter appearance. However, cheese yield and fat and water retention in the curd were affected and concomitantly cheese texture (A. Lemay et al., J. Dairy Science, 1994, 77:2870-2879). In still another process, separated butter oil was heat treated in order to destroy the β-carotene. This treatment may lead to production of toxic oxysterols (J.H. Nielsen et al., 1996, 63, 615-621). To overcome the above-mentioned problems the present invention discloses the use of lipoxygenases in dairy products to bleach or whiten them.

We have surprisingly found that only small amounts of lipoxygenases have a whitening effect on dairy products such as milk, cheese, butter oil, cream, whey products. By dairy products is meant products that contain at least 10% w/w%, preferably at least 30 w/w%, more preferably at least 50 w/w%, still more preferably at least 70 w/w% or most preferably at least 80 w/w% on dry solid basis of components of milk, preferably cow's milk. Components of milk are for example fats, proteins etc. As discussed above milk, especially cow's milk may naturally contain colouring compounds such as β-caroteen. It is believed that the mechanism of bleaching by lipoxygenase is based on the oxidative transition of double bonds in β-carotene (or other carotenoides or other compounds having double bounds) by radicals produced in the reaction of lipoxygenase and linole(n)ic acid. We have found the present enzymatic whitening does not result in off-flavours that might affect the perception of the treated dairy product. This whitening effect of lipoxygenase in dairy products is surprising when one realizes that the enzyme is present in the aqueous phase whereas the fatty acids are present in fat

particles of the dairy product. Also the β-carotene which is present in cows milk is present in the fat phase.

Soy derived lipoxygenase has been used for bleaching purposes in wheat and maize flour or in such applications as noodles (see e.g. JP61257158, JP53024039, JP56131358, JP53062946), but its use for cheese whitening has not been described.

Lipoxygenase is commercially available. Soy is a well-known source of lipoxygenase. Other sources include wheat varieties like durum, pea, faba beans, rice and lentils. Lipoxygenase may also be produced fermentatively. B. Knust (1990) Proc. 15th fermentively Int. Conf. Yeast. Gen Mol. Biol. p. S429 describes the use of baker's yeast for secreting legume lipoxygenase. Also, WO 02/20730 describes the production of lipoxygenase by the fungus Gaeumannomyces graminis, the identification of a lipoxygenase encoding gene from this organism and attempts to produce the functional protein in various microbial hosts.

Advantageously, the lipoxygenase is added to the milk before the cheese making process or other milk conversion or separation process starts. The lipoxygenase can also be added at a later stage, for example during the cheese making at the same time as the addition of rennet. Alternatively, the lipoxygenase can be added onto the cheese after cheese manufacturing. Also the lipoxygenase can be added to the whey or to the products obtained from whey such as whey protein or whey hydrolysates.

It will be appreciated that the skilled person in the art can easily determine the amount of LOX necessary to whiten the dairy product. In general 10 to 1000 units of LOX per gram of dairy product, preferably 50 – 500 units LOX per gram of dairy product, are used to bleach the dairy product.

Example 1

Determination of lipoxygenase acivity.

Lipoxygenase from soy will catalyze the oxidaton of lipids containing a cis,cis-1,4-pentadiene structure such a linoleic acid. The LOX activity is determined at pH9.0 at 25 ^{0}C using linoleic acid as the substrate. One unit will cause an increase per minute of 0.001 absorbance units at 234 nm (1 cm path length).

Example 2

Preparation of Lipoxygenase

Lipoxygenase was obtained from Sigma. Alternatively, lipoxygenase was prepared from soy bean whey water, obtained from Protein Technology International (Ieper, Belgium). A solid/liquid separation was performed over a Z-2000 filter plate, followed by a germ filtration over a Z-200 Schenk filter plate. This resulted in a clear soy whey water preparation, which was subsequently ultra-filtrated at pH8.2 on a 10 kD membrane. The final preparation contained 6500 lipoxygenase units or more, the precise number varying per batch of soy whey.

Example 3

Preparation of mini cheeses

Miniature cheeses were produced as described by Shakeel-Ur-Rehman et al. (Protocol for the manufacture of miniature cheeses in Lait, 78 (1998), 607-620). Raw cows milk was pasteurised by heating for 30 minutes at 63°C. The pasteurised milk was transferred to wide mouth plastic centrifuge bottles (200mL per bottle) and cooled to 31°C. Subsequently, 0.72 ml of starter culture DS 5LT1 (DSM Gist B.V., Delft, The Netherlands) was added to each of the 200 ml of pasteurised milk in the centrifuge bottles and the milk was ripened for 20 minutes. Than, $CaCl_2$ (132 µL of a 1 $mol.L^{-1}$ solution per 200mL ripened milk) was added, followed by addition of the coagulant (0.04 IMCU per ml). In case the experiment involved the use of lipoxygenase, this enzyme was added together with the coagulant. The milk solutions were held for 40-50 minutes at 31°C until a coagulum was formed. The coagulum was cut manually by cutters of stretched wire, spaced 1 cm apart on a frame. Healing was allowed for 2 minutes followed by gently stirring for 10 minutes. After that, the temperature was increased gradually to 39°C over 30 minutes under continuous stirring of the curd / whey mixture. Upon reaching a pH of 6.2 the curd / whey mixtures were centrifuged at room temperature for 60 minutes at 1,700*g*. The whey was drained and the curds were held in a water bath at 36°C. The cheeses were inverted every 15 minutes until the pH had decreased to 5.2-5.3 and were then centrifuged at room temperature at 1,700*g* for 20 minutes. After further whey drainage the cheese bleaching was determined.

Example 4
Determination of colour and bleaching

Mini cheeses were scanned on a colour scanner (Hewlett Packard Scanjet ADF) and analysed using the programme LabSMART (LabSMART, LLC, Logan Utah, USA). Colours were quantified with three parameters: L-factor(black =0 to white= 100), a-factor (green = -60 to red = +60) and b-factor (Blue = -60 to Yellow = +60). In addition, cheeses were evaluated visually in comparison experiments (control vs experimental) for whiteness.

CLAIMS

1. Process for the whitening of a dairy product which comprises adding lipoxygenase (LOX) to the dairy product.

2. Process according to claim 1 wherein the addition of LOX takes place before, during or after the production of the dairy product.

3. Process according to claim 1 or 2 wherein from 10 to 10000 units of LOX/g is used in or on the dairy product.

4. Use of LOX to bleach a dairy product.

5. A dairy product which is bleached by LOX.

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BLEACHING OF DAIRY PRODUCTS

ABSTRACT

The present invention describes a proces for the whitening of a dairy product, which comprises adding lipoxygenase (LOX) to the dairy product.